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PHENOTYPING FUSARIUM HEAD BLIGHT RESISTANCE IN OATS HAVING LOW DEOXYNIVALENOL CONTENT

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Doctoral dissertation

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ABSTRACT

Fusarium head blight (FHB) disease and the mycotoxins produced by its causal agents such as *Fusarium graminearum* Schwabe, *F. culmorum* (Wg. G. Sm.) and *F. langsethiae* Torp and Nirenberg have become a growing problem for oat (*Avena sativa* L.) production in the northern countries over the last decades. Since Nordic oats and oat products are branded as high quality and healthy food, FHB has to be managed. Controlling FHB by agricultural or manufacturing practises can be cumbersome. However, development of resistant cultivars would offer a highly needed and economical solution to the problem. Disease resistance refers to the plant's ability to either stop or slow down the progress of a disease. Breeding for resistance is of high priority in oats, but information on suitable phenotyping methodology and resistance sources against *Fusarium* species is scarce and dispersed. A phenotype is the expression of a certain genotype in a specific environment and phenotyping is measurement of phenotypes, which is a crucial part of plant breeding. In plant breeding, the variation within a plant species is utilized through crossings of individuals and selects plants from the progeny. By selection, traits of new plants such as productivity or disease resistance can be improved.

The primary aim of this thesis was to improve the tools for FHB resistance breeding in oats. A literature review was conducted focusing on resistance sources and phenotyping techniques available for oats against the most common deoxynivalenol (DON) mycotoxin producing *Fusarium* species, *F. graminearum*. The literature survey covered improved inoculation and screening methods as well as resistance and association studies. Several traits that may be related to resistance and used for resistance phenotyping were identified. Also, potential resistance sources were identified among both advanced cultivars and genebank accessions.

Resistance among genebank accessions and Nordic oat breeding material, inoculation methods and potential traits to measure resistance were evaluated in several field and greenhouse experiments in Finland. DON-producing *Fusarium* fungi were inoculated with spawn and spray methods to screen oat genotypes. *Fusarium*-infected kernels, germination capacity, and DON accumulation were compared as resistance related traits. A screening method relying on point inoculation was applied to study *Fusarium* infection in oats at a spikelet level. Agronomical and morphological traits that can potentially lead to reduced FHB infection were also phenotyped from the field trials and their relation to mycotoxin accumulation was investigated.

Greenhouse and field research resulted in significantly different oat genotype susceptibility rankings for both DON accumulation and *Fusarium*-infected kernels. Cultivars, breeding populations, and selected genebank accessions all represented high ranges of variation within DON accumulation. Earliness, height and flowering traits all significantly affected *Fusarium* infection and DON accumulation in the field conditions. When different groups of inoculated oat genotypes were investigated, both

cleistogamic and highly open flowering oats were found lacking high mycotoxin contaminations.

Interesting differences in FHB resistance were found. Especially, the most resistant and the most susceptible lines of the core set of 30 genotypes can work as sources for resistance as well as good checks for resistance evaluation. Correspondence between genotype rankings in *Fusarium* infection, DON accumulation, and loss of germination were found, but there were also genotypes that were susceptible based on one trait and moderately resistant based on the other. In greenhouse conditions, the spray-inoculated landrace VIR7766 was significantly more resistant against initial infection and DON accumulation than the rejected variety BOR31, but no difference in *F. graminearum* biomass measured in real-time quantitative PCR from spikelets at 6 days after point inoculation.

The results obtained from the field experiments had more practical relevance in identification of cultivars that accumulate less DON, because in the field conditions, the escape mechanisms such as early flowering, height or high rate of anther extrusion through open flowering can all contribute to DON accumulation and *Fusarium* infection within an oat plant. However, greenhouse research may have value for identification of novel resistance sources from unadapted germplasm. Different results for point and spray inoculation methods used in greenhouse indicate that once the infection is established, the role of resistance may be insignificant, since infection proceeded equally in moderately resistant and in susceptible genotypes. Large variation in several *Fusarium*-associated traits among different oat genotypes indicates that FHB resistance in Finnish oats can be improved. Further research with more specified hypotheses are recommended in order to better understand the genetics of FHB resistance in oats and also to achieve more efficient methods for the phenotyping of large progenies.

ABSTRAKTI

Punahome ja punahometoksiinit, joita tuottavat mm. seuraavat punahomelajit *Fusarium graminearum* Schwabe, *F. culmorum* (Wg. G. Sm.) ja *F. langsethiae* Torp and Nirenberg muodostavat kasvavan uhan pohjoisten alueiden kaurantuotannolle. Pohjoismainen kaura ja kauratuotteet on brändätty korkealaatuisiksi tuotteiksi, joten punahomeista aiheutuvat laatuongelmat on pidettävä hallinnassa. Taudinkestävät lajikkeet toisivat tarvittavan lisän olemassa oleviin taudin hallintakeinoihin, sillä haastellisina vuosina EU:n asettama raja-arvo deoksinivalenoli (DON) toksiinille voi ylittyä, vaikka kaikki muut hallintakeinot olisivatkin huomioitu. Taudinkestävyydellä eli resistenssillä tarkoitetaan kasvin kykyä joko pysäyttää tai hidastaa infektion etenemistä. Kestävyysjalostus koetaan tärkeäksi kauralle, mutta tieto sopivista fenotyyppaustekniikoista ja kestävyyslähteistä punahometta aiheuttavia *Fusarium* -lajeja kohtaan on ollut vähäistä ja hajanaista. Kasvinjalostaja luo risteytysten kautta uusia yhdistelmiä kasvilajin sisäisestä vaihtelusta ja pyrkii valitsemaan jälkeläisistä parhaimmat, mikä johtaa parannuksiin esimerkiksi tuottavuudessa ja taudinkestävyydessä. Fenotyyppien määrittäminen, eli fenotyyppaus, on keskeinen osa kasvinjalostusta. Fenotyyppi on tietyn perimän omaavan yksilön eli genotyypin ilmentymä tietyssä ympäristössä eli esimerkiksi kuinka vakava tietyn jalostuslinjan tartunta on tietyssä kokeessa.

Tämän väitöskirjan ensisijainen tavoite oli parantaa punahomeen kestävyysjalostuksen tilaa kauralla. Kirjallisuuskatsauksella selvitettiin, millaisia tekniikoita ja kestävyyslähteitä *F. graminearum* -punahomeella on saatavilla. *F. graminearum* on DON-toksiinia tuottavista punahomeista yleisin. Kirjallisuudesta nousi esiin hyödyllisiä tartutusmenetelmiä, havainnoitavia ominaisuuksia sekä tietoa kestävyyden periytymisestä kauralla. Sekä jalostus- että geenipankkiaineiston arvioitiin sisältävän potentiaalisia kestävyyden lähteitä.

Geenipankki- ja jalostusaineistojen resistenssiä, tartutusmenetelmiä ja lupaavia resistenssiä kuvaavia ominaisuuksia lähdettiin arvioimaan useiden kenttä- ja kasvihuonekokeiden avulla. Kokeissa tartutettiin DON-toksiinia tuottavia punahomeita eri menetelmin kauroihin. Tartunnan saaneita jyviä, itävyyttä ja DON-pitoisuutta vertailtiin resistenssiä mittaavina ominaisuuksina. Pistetartutukseen nojaavaa menetelmää käytettiin sieni-infektion voimakkuuden tarkasteluun tähkylätasolla. Monet agronomiset ja morfologiset ominaisuudet voivat myös vaikuttaa punahometartunnan määrään, ja siksi myös näiden fenotyyppaukseen keskityttiin peltokokeissa, ja niiden vaikutusta mykotoksiinien kertymiseen tarkasteltiin.

Sekä DON-toksiinipitoisuuksien ja tartunnan saaneet jyvien tapauksessa kasvihuone- ja kenttäkokeet luokittelivat kauroja hyvin eri tavoin kestävistä alttiisiin. DON pitoisuuksissa oli suurta vaihtelua riippumatta siitä

tarkasteltiinko lajikkeita, jalostuslinjoja vai geenipankkikauroja. Aikaisuus, pituus ja kukintaominaisuudet kaikki vaikuttivat merkitsevästi punahometartuntojen määrään ja DON pitoisuuksiin kenttäkokeissa. Kukintatyypiltään joko erittäin suljettu tai avoin kauralajike vältti kokeissamme korkeimmat toksiinipitoisuudet.

Kaurojen punahomekestävyydessä löytyi mielenkiintoisia eroja. Varsinkin kaikista poikkeavaisimmat genotyypit tarkimmin fenotyypitetyssä 30 kauran joukossa voivat toimia sekä kestävyyden lähteinä että hyvinä verrokkeina kokeissa. DON, tartunnan saaneet jyvät ja itävyys korreloivat osittain keskenään, mutta edustavat myös omia kestävyyskomponenttejaan. Kasvihuoneessa sumutartutettu maatiaslajike VIR7766 oli merkittävästi kestävämpi tartuntaa ja DON pitoisuuden nousua kohtaan, kuin hylätty lajike BOR31. Sen sijaan, kun tartuke pipetoitiin tähkylän sisään, se oli tuottanut yhtä paljon punahomeen biomassaa molemmissa genotyypeissä kuusi päivää tartutuksesta.

Kenttäkokeiden tulokset olivat paremmin sovellettavissa tilanteeseen viljelijöiden pelloilla ja ne ovat käyttökelpoisempia tulevaisuuden lajikkeiden DON-pitoisuuksien alentamisessa. Kasvihuonetutkimukset kuitenkin voivat mahdollistaa kontrolloituine olosuhteineen tutkimuksen ja mm. pohjoisiin olosuhteisiin sopeutumattomien kestävyyslähteiden vertailun. Kenttäkokeissa infektion todennäköisyyttä pienentävät mekanismit, kuten aikainen kukinta, pitkä korsi tai korkea heteiden ulostyöntyminen avoimen kukinnan myötä lisäävät kauran kestävyyttä punahometartuntaa vastaan. Kasvihuoneessa tehdyistä piste- ja sumutartutuksista saadut toisistaan poikkeavat tulokset viittaavat, ettei tartunnan tapahduttua kestävyyden rooli olisi merkittävä. Suuri muuntelu useissa punahomeeseen yhdistettävissä ominaisuuksissa eri kauragenotyypeissä viittaa suomalaisten kaurojen punahomekestävyyden olevan parannettavissa fenotyyppauksen avulla. Jatkotutkimuksia tullaan tarvitsemaan, jotta kestävyyden periytyvyyttä voitaisiin paremmin ymmärtää kauralla ja, kasvinjalostukselle tyypillisten suurten jälkeläistöjen fenotyyppauksesta saataisiin kustannustehokkaampaa.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications and manuscripts:

I Hautsalo, J., Jalli, M., Manninen, O., Veteläinen, M. (2018). Evaluation of resistance to *Fusarium graminearum* in oats. Euphytica 214:139. doi: 10.1007/s10681-018-2222-3

II Hautsalo, J., Jauhiainen, L., Hannukkala, A., Manninen, O., Veteläinen, M., Pietilä, L., Peltoniemi, K., Jalli, M. Resistance to Fusarium head blight in oats based on analyses of multiple field and greenhouse studies. European Journal of Plant Pathology. doi: 10.1007/s10658-020-02039-0

III Hautsalo, J., Latvala, S., Hannukkala, A., Manninen, O., Haapalainen, M., Jalli, M. Two oat genotypes with different field resistance to Fusarium head blight respond similarly to 1 the infection at spikelet level. Manuscript submitted to Journal of Plant Pathology.

IV Herrmann, M.H., Hautsalo, J., Georgieva, P., Bund, A., Winter, M., Beuch, S. (2020) Relationship between genetic variability of flowering traits and Fusarium mycotoxin contamination in oats. Crop Science 60: 852-862. doi: 10.1002/csc2.20125

The publications are referred to in the text by their roman numerals.

AUTHOR'S CONTRIBUTION

I Evaluation of resistance against *Fusarium graminearum* in oats.
Juho Hautsalo (JH), Marja Jalli (MJ), Outi Manninen (OM) and Merja Veteläinen (MV).

JH wrote the first version of the manuscript. The other three authors contributed equally to the preparation of the manuscript by making suggestions about what information should be included and what excluded and by revising chapters. MV and JH organized funding for this study.

II Resistance to Fusarium head blight in oats based on analyses of multiple field and greenhouse studies.
Juho Hautsalo (JH), Lauri Jauhiainen (LJ), Asko Hannukkala (AH), Outi Manninen (OM), Merja Veteläinen (MV), Leena Pietilä (LP), Kirsi Peltoniemi (KP) and Marja Jalli (MJ)

JH contributed to the initial idea, to the experimental design and analysis of results. JH carried out the field experiments at Laukaa site on years 2016 and 2017 (planting, inoculations, irrigation, pest and disease management, phenotyping, harvesting). JH wrote the first draft and contributed to the subsequent versions of the manuscript. JH also contributed to the data-analyses. LJ contributed to the original idea, conducted the statistical analyses and participated to the writing of the manuscript. AH contributed to the original idea and commented the manuscript several times. MV contributed to the original idea, helped to revise the manuscript OM contributed to the original idea and helped to revise the manuscript. MV was also the project manager for the project where data was gathered and analysed. LP contributed to the original idea, designed the field experiments and provided the phenotype data for the analysis. She also read and commented the manuscript. KP contributed to the original idea, was responsible for the mycotoxin analyses and also commented on the manuscript. MJ contributed to the original idea and provided data for the analysis. She also designed the greenhouse experiments, contributed to the design of field experiments and managed the analyses of yield samples excluding toxins. In addition MJ helped to revise the manuscript and organized funding for part of the work.

III Two oat genotypes with different field resistance to Fusarium head blight respond similarly to 1 the infection at spikelet level.
Juho Hautsalo (JH), Satu Latvala (SL), Outi Manninen (OM), Minna Haapalainen (MH), Asko Hannukkala (AH) and Marja Jalli (MJ)

JH contributed to the initial idea, designed the point inoculation experiments and conducted the point inoculations in greenhouse. JH also took part to the analysis of real time PCR results and made statistical analyses for the manuscript and wrote the first version of the manuscript. SL conducted

isolation of dna, developed the PCR protocol and made the real-time PCR analyses together with MH and contributed to the writing of the manuscript. OM contributed to the initial idea and helped to revise the manuscript. AH helped to revise the manuscript and contributed to statistical analyses. MJ contributed to the initial idea, designed and organized the greenhouse spray inoculations and arranged their analyses and helped to revise the manuscript. JH and MJ both applied funding for this work.

IV Relationship between genetic variability of flowering traits and Fusarium mycotoxin contamination in oats.
Matthias Heinrich Herrmann (MH), Juho Hautsalo (JH), Adalbert Bund (AB), Mark Winter (MW), Steffen Beuch (SB) and Paulina Georgieva (PG) .

MH, MW, AB and JH contributed to the initial ideas. MW coordinated experiments related to panel_2, PG performed all experiments in Goettingen, SB coordinated the experiments in Boehnshausen with oat panel_2, AB conducted experiments in Freising with oat panel_1. MH coordinated the experiments, conducted and phenotyped the experiments in Gross Luesevitz, conducted statistical analysis and wrote the first version of the manuscript. JH was responsible for the establishment, management and phenotyping of field experiments in Finland on years 2016 and 2017 (oat panels_1 and 3). JH also calculated anther retentions from the samples of these experiments, arranged the DON analyses for them and determined germination capacities and *Fusarium* infected kernels from all sites on the experiment conducted on 2017. All authors helped to revise the manuscript.

ABBREVIATIONS

AE	anther extrusion
AR	anther retention
BOR	acronym referring to Boreal Plant Breeding Ltd.'s breeding lines
DON	deoxynivalenol
e.g.	exempli gratia
EU	European union
FHB	Fusarium head blight
FIK	Fusarium infected kernels
GC	germination capacity
i.e.	id est
OFL	rate of open flowering
PCNB	pentachloronitrobenzene
PCR	polymerase chain reaction
QTL	quantitative trait locus (or loci)
VIR	acronym referring to the accessions from N. I. Vavilov All-Russian Institute of Plant Genetic Resources
VYR	Finnish cereal committee

1 INTRODUCTION

1.1 NORDIC OATS

Nordic conditions favor production of oats (*Avena sativa* L.) for feed and food. Oat is a crop that has relatively little disease problems and, due to its vigorous growth, it competes well with most weeds (Marshall et al. 2013). Thus, oat is favoured in crop rotation; it is often grown in less productive fields even though the utilisation of yield potential of modern cultivars requires investments in modern agronomical technology in good soils. Oat suits well also for organic production. In human consumption, oat can be seen as health-benefiting crop; especially oat fibre content is appreciated and valued in morning cereals and other oat-based food products. The health claims for beta-glucans and their positive effects to maintain normal blood cholesterol concentrations have been approved by the EU (EFSA 2009 and 2011). Use of oats for human use is growing steadily and new oat products are being developed for the consumer market. For example baking technology for oat bread has been recently improved, allowing the consumers enjoy breads that are made entirely from this gluten free cereal (Flander et al, 2007). Also plant-based milk alternatives such as oat drinks are a rising trend (Sethi et al. 2016). Nordic oats has become a brand of high quality cereal marketed by Finnish mill industry (Fazer mills 2019).

Approximately 1 million tons and nearly 30 percent of the yearly cereal production in Finland is oat (Luke 2019). During recent years (2015-2019), the cultivated area for oats has been between 306 500 and 337 000 hectares, which is between 13.5 and 17 per cent of total agricultural land use in Finland (Luke 2019). Over 300 000 tons of yearly export make Finland one of the most important oat producers in Europe and the second largest exporter in the world after Canada (FAOSTAT 2017). In Finnish fields, oat is a common crop in almost all regions below Lapland, but the most common oat production areas are coastal regions of Finland such as Ostrobothnia (Luke 2019, Finnish Cereal Committee 2019a). Sixty-six oat cultivars were listed National List of Plant Varieties in 2018 (Finnish Cereal Committee 2019b). The five most commonly grown cultivars, which are all blonde hulled, are: Niklas, Meeri, Belinda, Matty and Akseli. They account approximately 40 % of all oat acreage in Finland (Finnish Cereal Committee 2019b).

1.2 FUSARIUM HEAD BLIGHT IN OAT

Fusarium diseases such as fusarium head blight (FHB) or fusarium crown rot cause severe losses in cereal production globally (Buerstmayr et al. 2014, Liu et al. 2015, Nganje et al. 2004, Bjørnstad & Skinnes 2008) and these are especially harmful for farmers and for the entire grain chain. FHB in oat can cause yield losses (Kiecana et al. 2002), low seed germination (Tekle et al. 2013), and accumulation of mycotoxins (Scott 1989). Mycotoxins, especially toxigenic sesquiterpene epoxides, the trichothecenes, are the most severe problems related to FHB. The harmful impacts of mycotoxins to human and animal health have led to limitation of these substances in food and cereals. In EU, the concentration of one of the most common mycotoxins, deoxynivalenol (DON), has been limited to 1750 μg/kg in unprocessed oat grains (European Commission Regulation No 1881/2006). There is also a recommendation for feed usage (<7500 μg/kg, Lantmännen Agro 2019). In addition, there is a continuous discussion of setting thresholds for other mycotoxins, such as T-2 and HT-2 mycotoxins, which are produced by *Fusarium* species such as *F. langsethiae* Torp&Nirenberg, *F. sporotrichioides* Sherb. Sacc., and *F. sibiricum* (Yli-Mattila et al. 2004a, Yli-Mattila et al. 2008, Jestoi et al. 2008, Yli-Mattila et al. 2011). Since the thresholds are used as reasons to either discard or lower the price of the oat lot, it is relevant to express that FHB is a major concern in both aspects, in health and economy.

Mainly due to changes in cultivation practises and climate, the most important producer of DON mycotoxin and one of the most important fungal plant pathogens in the world (Dean et al. 2012), *Fusarium graminearum* Schwabe has increased in westernEurope and also in northern America during the recent decades (Tekauz et al. 2000, Tekauz et al. 2008). The incidence of *F. graminearum* is also increasing in northern Europe (van der Lee et al. 2015) and it has recently become the most common species associated with DON production in Nordic countries (Hofgaard et al. 2016a, Hietaniemi et al. 2016, Fredlund et al. 2013). In Finland, *F. graminearum* was determined to explain DON contaminations in cereals more *than F. culmorum* (Wm. G. Sm.) and *F. langsethiae* was determined to explain T-2/HT-2 contaminations more than *F. sporotrichioides* (Yli-Mattila et al. 2009, Kaukoranta et al. 2019).

An effective management of FHB and DON in oat is difficult due to several reasons. First of all, in addition to *F. graminearum*, the disease is caused by several different *Fusarium* species that favour different environmental conditions. FHB is mainly caused by 16 different species belonging to the so-called *F. graminearum* species complex, which has a variable constitution between environments (van der Lee et al. 2015). These species can produce several different mycotoxins. Most typical of these in Finland, in addition to *F. graminearum sensu tricto*, are *F. avenaceum* (Fr.) Sacc., *F. culmorum*, *F. langsethiae*, *F. poae* and *F. sporotrichioides* Sherb.

Sacc. and *F. tricinctum* (Corda) Sacc. (Yli-Mattila et al. 2004b, Hietaniemi et al. 2016). Secondly, the mycotoxin production and the infection by *Fusarium* species in cereals are influenced by several overlapping but not identical external factors. Moisture is the most important factor (Lacey et al. 1999). Other factors include factors such as temperature (Brennan et al. 2005), light intensity, nitrogen fertilization (Doohan et al. 2003), cultivation practices (Yli-Mattila et al. 2009, Kaukoranta et al. 2019) and cultivar susceptibility (Wegulo et al. 2015). For example, dry and warm conditions favour *F. poae*, warm and humid environments *F. graminearum* and cool and rainy conditions favour *F. culmorum* (Xu et al. 2008). Moreover, the infections by DON producers are promoted by moisture during anthesis, but the mycotoxin accumulation is also dependant on the weather after flowering (Kaukoranta et al. 2019, Hjelkrem et al. 2016). In contrast to DON producers, *F. langsethiae* has an earlier and smaller response to moisture before anthesis and strongly benefits from warm autumn weathers (Kaukoranta et al 2019).

The management of FHB in cereals requires an integrated disease management approach including crop rotation system, cultivar, fungicide treatment, weed management and soil tillage (Hietaniemi 2016, Wegulo et al. 2015). In addition, post-harvest management of the harvested crop plays an important role in the prevention of mycotoxin accumulation (Magan & Aldred 2007). Several fungicides are registered to control the disease, but timing of spraying is critical and even rightly timed spraying may not protect from infection. There exists continuous infection pressure from airborne spores (Keller et al. 2013) from other fields and crop residues (Sutton 1982). Also a longer flowering period in oats compared to wheat increases susceptibility (Misonoo 1936, Percival 1921). Under high infection pressure oats can accumulate more DON than wheat or barley and creates a concern for industry promoting oat-based foodstuffs. More resistant oat cultivars would bring awaited contribution for the disease management (Hietaniemi 2016).

1.3 EPIDEMIOLOGY OF FHB

Most FHB pathogens, especially the DON producing *Fusarium* fungi, infect wheat and barley spikes and oat panicles around anthesis (Schroeder and Christensen 1963; Wagacha and Muthomi 2007, Tekle et al. 2012). FHB-causing pathogens overwinter in crop residue as mycelium and resting spores (clamydospores) or in infested seeds (Parry et al. 1995). A range of alternative hosts, including maize, soybean, sorghum, wild oats, and various common weeds, have been reported to be sources of *F. graminearum* inoculum (Sutton, 1982; Fernandez, 1991, Dill-Macky and Jones, 2000, Pereyra et al. 2008). Crop residue is considered to be the main source of inoculum for *F. graminearum* (Sutton 1982). Increase of crop debris as well

as lack of crop rotation have been shown to increase disease severity in wheat (Dill-Macky and Jones 2000). The relationships between mycotoxin accumulation, crop rotation and tillage practises may, however, be different in different *Fusarium* species and in different regions (Kaukoranta et al. 2019). *F. graminearum* can produce perithecia and ascospores on soil surface for two to three years after the host crop has been harvested (Khonga and Sutton 1988). This saprophytic survival of *F. graminearum* in crop residue determines the primary inoculation load to growing oat plants.

FHB spreads from inoculum to crop by air movement, which is the main pathway for *F. graminearum* sexual ascospores, or with rain splashes, which is more typical for conidia (Sutton 1982). Since the most of FHB causing species do not have sexual forms, the splash dispersal of conidia is a very important mechanism. Different spreading mechanisms were found to result in different deposition patterns in wheat canopy (Manstretta et al. 2015), which indicates that conidia will need to use nodes and leaves as bridges where sporulation cycle or further splashes are needed before reaching the head (Parry et al. 1995). The long-distance spread of *F. graminearum* is likely to occur via transportation of infected seeds, although viable ascospores of *F. graminearum* are estimated to disperse via air movements from tens to hundreds of kilometres (Keller et al. 2013). When infected seeds are planted, seedling blight, Fusarium crown rot and and Fusarium root rot can develop in the following season (Tekle et al. 2013, Parry et al. 1995) and from these sources of inoculum the disease can spread to heads and panicles.

Fusarium spores are more likely to land on outer floral parts than inside the florets of oats (Tekle et al. 2012, Xue et al. 2015) and thus they have to grow hyphae towards the soft floral interior tissue. In inner surfaces of the flowers, they can form infection structures such as infection cushions and foot-like structures (appressoria) (Boenisch and Schafer 2011, Divon et al. 2019) which can penetrate cell walls and produce mycotoxins. Degrading anthers are suggested to act as a route into the flower since they are often colonized first (Tekle et al. 2012). If the infection occurs during flowering the colonization of developing ovary may easily lead to dead and empty grain (Tekle et al. 2012). Infection continues until maturity and secondary infections can occur up to yellow maturity (Tekle et al. 2013).

1.4 ACCUMULATION OF DON

Trichothecene production may be a major survival factor for the fungus and for example DON has shown antimicrobial properties in crop residue (Bennett and Klisch 2003). The ability to produce DON in wheat stubble is shown to correlate with the aggressiveness of *F. graminearum* isolates during the infection (Tunali et al. 2012). Trichothecene production has been shown to contribute pathogenicity of *F. graminearum* in several cereal species (Langevin et al 2004). DON mycotoxin disrupts protein synthesis by

binding into ribosomes. The problems with protein synthesis reduce cell division, cause oxidative stress, and eventually accumulation of DON kills the plant cells if the plant is not able to remove the toxin or detoxify it (Audenaert et al. 2013). There is a growing evidence found mainly in wheat that *F. graminearum* uses DON as a weapon against the host plant in several phases of infection (Gunupuru et al. 2017, Walter et al. 2010) and that resistant genotypes include genes that can contribute to removal or transformation of DON. Several detoxified forms for DON such as DON-3-O-glucoside or 16-hydryxy-DON are known (Gunupuru et al. 2017). During detoxification DON binds to hydrophilic molecules such as glucose and then transported out from the cytoplasm.

Large-scale DON production does not usually start until the pathogen is challenged by the host defence mechanisms (Walter et al 2010; Audenaert et al. 2013). In wheat, many defences of the host plant against the infection actually promote trichothecene synthesis, such as the sucrose that is transported to infected tissue as energy source to fight the disease (Jiao et al. 2008) and stress-related polyamine production (Gardiner et al. 2009). An important phase in the SA defence pathway, the oxidative burst increasing H2O2 level in the plant tissue, is actually found to promote early DON synthesis in vitro (Ponts et al. 2007; Audenaert et al. 2010). This may be a way for the fungus to halt the formation of defence proteins such as chitinases, peroxidases and PR-proteins (Audeanaert et al. 2013).

DON has been speculated to act as immunosuppressor in the beginning of infection, preventing programmed cell death (Diamond et al. 2013), which aids the fungus to feed itself on living plant tissue and to establish an infection. Additionally, DON has been also associated with delayed activation of plant defence response pathways, based on gene expression patterns between susceptible and resistant wheat genotypes inoculated with either a wild type or toxin deficient mutants of *F. graminearum* (Foround et al. 2012). Recognition of a pathogenic microbe such as *Fusarium* through microbe-associated molecular patterns (MAMP, Newman et al. 2013) could lead into the formation of thickened cell walls (papillae) under the penetration structure, segregation of antifungal compounds such as chitinases and induction of defence signalling (Walter et al. 2010). Trichothecene deficient mutants have no limitations in their ability to penetrate wheat floral tissue, suggesting that DON has no major role in the beginning of infection (Boenisch and Schafer 2011). This applies also in barley where no DON was detected from infected tissue until 48 h after inoculation (Evans et al. 2000).

1.5 RESISTANCE AGAINST FUSARIUM HEAD BLIGHT

Mycotoxins accumulate during infection; this is not direct consequence of the presence of fungus, but more likely a consequence of different interactions

between environment, host and the pathogen (Walter et al. 2010). Moreover, the infection of a floret, depending on the developmental stage of the host, causes damage and in the worst case dead of the germ. If this occurs at flowering the grain can remain empty (Bjørnstad & Skinnes 2008, Bjørnstad et al. 2017). Damage, mycotoxin and the presence of the fungus can reduce germination ability (Tekle et al. 2013), which are especially risky for seed producers.

In cereals, the resistance against FHB is commonly divided into five components: resistance against initial infection (type I), resistance against the spread of infection (type II, Schroeder and Christensen 1963), resistance to toxin accumulation (type III, Miller et al. 1985), resistance against kernel infection and tolerance (types IV and V, respectively, Mesterházy 1995, Mesterházy et al. 1999). Resistance can also be either passive or active, depending on whether the resistance mechanism requires presence of the pathogen to be triggered (Buerstmayr & Buerstymayr 2014), e.g. the height of the plants and their flowering time can both lead to avoidance of fungal spores and in that way reduce the mycotoxin accumulation in field trial conditions (Bjørnstad et al. 2017).

From over 200 known quantitative trait loci (QTL) for resistance against FHB in wheat about 50 % originate from Asian genotypes (Buerstmayr et al. 2009) and over half of these QTL confer type III resistance (Liu et al. 2009), the rest divide quite evenly among types I, II and IV. The function of these QTL is still under investigation. Even with the case of the most important FHB QTL in wheat, *Fhb1*, which originates from Chinese landrace Sumai 3, there exists three different hypotheses regarding its function (Su et al. 2019, Li et al. 2019, Rawat et al. 2016).

The genetics behind the resistance of oats are similarly quantitative as in wheat but only a few QTL have been identified (Bjørnstad et al. 2017). Several traits can be used to measure the resistance to FHB in oats, but these traits may not necessarily correlate well with each other. For example, FHB symptoms can be quantified but they correlate weakly with DON contents in oat, whereas germination and DON levels (Tekle et al. 2018) and *F. graminearum* DNA levels and DON correlate well (Yli-Mattila et al. 2009, Yli-Mattila et al. 2013). Despite the good correlation, there are some oat genotypes that had good germination even though their DON levels were high (Tekle et al. 2018), which indicates that mycotoxin contamination and the viability of kernels represent different resistance components. Several potential resistance sources have been identified for oats in multiple screening studies (Tekauz et al. 2008, Bjørnstad & Skinnes 2008, Gagkaeva et al. 2013, Bjørnstad et al 2017, Tekle et al. 2018).

Phenotyping FHB in plant breeding can be a challenge. A sufficient number of replications are required to quantify differences in resistance. For example, Tekle et al. (2018) used a minimum of four experiments in different environments for sufficiently reliable FHB resistance rankings. For this reason, in the early breeding generations with lots of variation and in with

small seed amounts available, phenotyping for FHB resistance is not feasible, even if the risk of losing all the seeds for infection would be ignored (Buerstmayr & Buerstmayr 2014). Another challenge in phenotyping is that the traits that give us the most reliable results for selecting resistant genotypes are expensive to measure. This is especially a problem for oat where unclear symptoms do not serve as a reliable screening method (Tekle et al. 2018).

2 AIMS OF THE STUDY

In this doctoral thesis the primary aim was to improve tools for resistance breeding in oats against *Fusarium* infection and deoxynivalenol accumulation in Finland. This aim can be further divided into three specific aims, which are:

I Gather, test, develop and choose phenotyping methods for selecting resistant oat genotypes.

II Investigate the role of flowering traits and other morphological traits in oat FHB resistance

III Evaluate resistance within Nordic oat breeding material and selected genebank accessions.

The following hypotheses were tested:

-Inoculated field experiments can separate FHB resistant oats from susceptible oats

-Inoculated greenhouse experiments can separate FHB resistant oats from susceptible oats.

-*Fusarium* infected kernels, germination capacity and DON accumulation are indicators FHB resistance

-Accumulation of *Fusarium graminearum* biomass and loss of plant tissue fresh weight can be used to separate FHB resistant oats from susceptible oats

-Morphological traits including height, earliness and flowering traits correlate with FHB resistance

-Nordic oat breeding material and genebank accessions both contain FHB resistance

3 MATERIALS AND METHODS

The key methods and plant material used in the thesis are provided in Table 1 and discussed in this chapter. More comprehensive descriptions of the materials and methods including statistical analyses conducted for each experiment can be found in Papers II, III, and IV. Paper I provides an overview of methodology described in literature.

Table 1. The methods and type of plant material applied in the original articles of this thesis.

Category	Description	Papers
Inoculation methods	Point inoculation	III
	Spray inoculation	II, III, IV
	Spawn inoculation	II, IV
Visual scoring:	Height	II, IV
	Earliness	II, IV
	Absence of hulls and colour of hulls	II
	Percentage of open flowering (OFL)	IV
	Anther extrusion (AE)	IV
Analyses	Mycotoxin accumulation (DON)	II, III, IV
	Fusarium infected kernels (FIK)	II, III, IV
	Germination capacity (GC)	II, IV
	Fusarium graminearum/oat DNA ratio	III
	Spikelet fresh weight reduction	III
	Anther retention (AR)	IV
Plant material	Genebank accessions	II, III, IV
	Breeding lines	II
	Cultivars	II, III, IV
Environment	Greenhouse	II, III
	Field	II, IV

3.1 PLANT MATERIAL AND DATA

The 348 Nordic breeding lines and 40 cultivars that were studied in Paper II were provided by Boreal Plant Breeding Ltd. In addition, 16 potentially resistant genebank accessions were provided for Dr. Outi Manninen from N. I. Vavilov All-Russian Institute of Plant Genetic Resources by Docent Tatiana Gagkaeva (Paper II). Dr. Matthias Herrmann (Julius Kühn-Institut) provided seeds for panels of 50 and 16 oats for the two separate experiments made in Paper IV. These panels included modern cultivars and older genebank accessions with potential differences in flowering habit as well as resistance to FHB. Studies in Papers II and III were done in Finland whereas Paper IV contained both

Finnish and German field experiments. Paper III used point and spray inoculation for a comparison of two contrasting oat genotypes in controlled greenhouse environment. Paper II studied resistance parameters and rankings in field and greenhouse experiments in a combined data-analysis of 13 field and 8 greenhouse experiments and Paper IV focused on studying flowering traits and FHB resistance parameters of oats in field experiments with multiple sites.

3.2 INOCULATION METHODS

Figure 1. Peritrechia formation on *Fusarium graminearum* spawn inoculum.

Three different types of inoculation methods were used in this Thesis (Paper I, Table 1). A point inoculation was conducted by inserting a *Fusarium* spore suspension inside a floret in mid panicle region by a pipette at anthesis. In the spray inoculation procedure, the inoculum suspension was sprayed over the plants at anthesis. The field inoculations in Papers II and IV were done with either spray or spawn inoculations depending on the experiment. All greenhouse experiments and the first field experiments in Paper II were spray inoculated and since 2015 spawn inoculation method was used in field. The spawn inoculation (Fig. 1) is based on autoclaved grains infested by several *F. graminearum* isolates (Skinnes et al. 2010, Tekle et al. 2018). This was spread evenly within the irrigated field nursery before panicle emergence. The experiment was then irrigated regularly to maintain moisture suitable for formation of peritrechia and ascospore release (Fig. 2). Individual isolates or mixtures of several *Fusarium* isolates isolated from Finland were used in the experiments in Finland (Papers II, III and IV). For the experiments made in Germany local isolates were used (paper IV).

3.3 VISUAL SCORINGS

Plant height in centimetres was measured from several field experiments in Papers II and IV. Additionally, visual estimates for plant height were grouped in three categories (short, intermediate and high) in Paper II. In Paper II, earliness was scored in days to heading or days to maturity or by scale from 1 to 5, where 1 was the earliest and 5 the latest maturing genotypes. Additionally, in Paper IV, earliness was estimated by determining the developmental stage of the genotypes twice with one week interval around panicle emergence. Hulless and dark hull colour were registered from the field trials containing genotypes from N. I. Vavilov All-Russian Institute of Plant Genetic Resources (Paper II).

Percentage of open flowers was scored during the afternoon hours when the 50 selected genotypes in 2016 and 16 selected genotypes in 2017 had started flowering (Paper IV). Upper and lower panicle parts were scored separately and each scoring was repeated at least twice during the flowering of the plots that were scored. Anther extrusions of the oat genotypes in Paper IV were determined from two replicate plots in the experiments. In each plot two transparent bags were put over four panicles per bag before flowering. The amount of anthers inside the bags was assessed after flowering on a scale from 1 (no anthers visible) to 9 (in 100% of spikelets anthers pushed out or collected in the bag).

3.4 ANALYSES OF SAMPLES AND DATA

Total of 2349 DON accumulation analyses, 4665 analyses of *Fusarium* infected kernels (FIK) and 1516 germination capacity (GC) analyses were analysed in Papers II and III. In addition GC and FIK analyses were made in one of the experiments in Paper IV and DON analyses from specific spikelet samples collected from the plots were made in that paper. A total of DON was measured by a serological assay (ELISA RIDASCREEN® Kits R5906, R-Biopharm) according to the manual. All DON analyses in Papers II, III and the DON analyses for the Paper IV's experiments conducted in Finland were made by Boreal Plant Breeding Ltd. FIK were calculated by placing samples of 50 kernels per plot on agar plates with a selective PCNB (pentachloronitrobenzene (Nash and Snyder medium, Nelson et al. 1983)) medium favouring *Fusarium* growth instead other bacteria and fungi. This is a method introduced by Parikka et al. (2007). GC was determined by paper testing samples of 100 seeds according to the international seed testing agency's instructions (ISTA 2006).

Relative amount of *F. graminearum* in plant by real-time PCR and spikelet fresh weight reduction were determined from 120 point inoculated spikelet samples at 6 days post inoculation (Paper III). For the calculation of relative *F. graminearum*/ plant DNA ratio, DNA was extracted, *Fusarium*

specific primers and oat reference gene primers were selected and real-time PCR reactions were run and analysed. Fresh weigh was calculated by reducing the weight of inoculated spikelet from an average spikelet weight of water inoculated controls.

Anther retention (AR) was determined in Paper IV from two replicated plots per experiment. Anthers were calculated from four florets from selected panicle locations from frozen samples of 10 panicles per plot.

The results from analyses were tested for statistical significance. Mostly a mixed model approach in SAS program was applied (SAS Institute Inc., Cary, NC, USA) and more comprehensive descriptions can be found from the Papers.

4 RESULTS

4.1 RESISTANCE-RELATED TRAITS IN THE FIELD AND GREENHOUSE

The estimates (Best Linear Unbiased Estimators=BLUE) for different FHB resistance-related traits including DON, FIK and GC measured in eight inoculated greenhouse, and 13 field, experiments (Paper II) separated highly susceptible genotypes from moderately resistant genotypes in both environments (Figures 2a-2d). Calculations for statistical power (Paper II) revealed that the least significant differences in DON accumulation between oat genotypes started to become feasible when data from more than three field experiments or two greenhouse experiments were combined. The core set of 30 oat genotypes that consists of cultivars, breeding lines and genebank accessions phenotyped in several field and greenhouse trials was formed to demonstrate the results (Fig. 2 and Paper II).

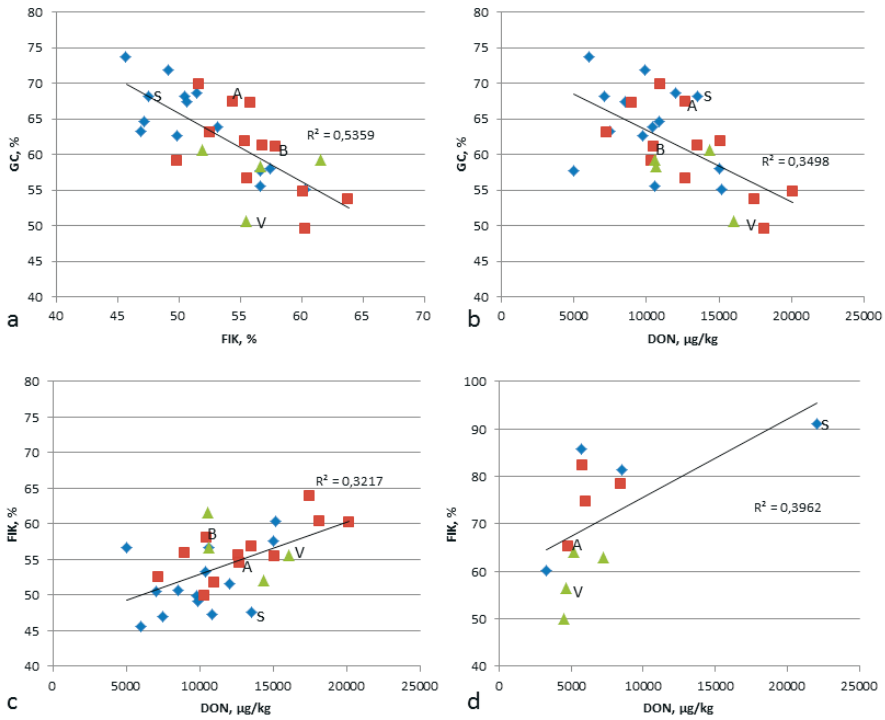

Figure 2. Best linear unbiased estimators (BLUEs) for DON accumulation (DON), *Fusarium* infected kernels (FIK), germination capacity (GC) plotted against each other for the core set of 30 oat genotypes. Different symbols represent different types of material, which are: breeding lines and a rejected variety (diamond), cultivars (square) and genebank accessions (triangle). Figures

2a, 2b and 2c consist from field BLUEs and Fig. 2d represents greenhouse estimates. Four genotypes are marked on the figures with letters on the right side of their symbols (Belinda=B, Akseli=A, susceptible BOR31=S and VIR7766=V). The lines and regression coefficients represent all points.

FIK, GC and DON were all identified as traits explaining variation of FHB resistance in oats. There was a significant correlation between the GC and the DON contents of field samples (r=-0.65, $P<0.0001$, Paper II). *Fusarium* inoculated oat genotypes with similar DON accumulation levels had clear differences in their GC, which will be discussed below together with other examples, where genotypes rank differently depending on the trait. In greenhouse experiments (Papers II and III), DON accumulation and FIK estimates correlated well (r=0.68, $P<0.001$, Paper II), but in in field the correlation at sample level was weak when all experiments and genotypes were considered. When estimates of genotypes with relatively high number of repeated measurements were considered (the correlations of the core set in Paper II), the correlations improved. Moreover, in Paper IV, where genotypic correlation between FIK and DON was calculated for the panel of 16 oats, the correlation was rather high (r=0.69, $P<0.01$).

4.2 ADDITIONAL TRAITS AND METHODS

Point-inoculated *F. graminearum* spore suspensions were shown to accumulate *F. graminearum* biomass (the relative amount of *F. graminearum* in plant, $P<0.001$) and reduce the fresh weight ($P<0.001$) of oat spikelets (Paper III). This method was not, however, able to distinguish two oat genotypes that gave continuously very different levels of DON and FIK in spray inoculated greenhouse experiments. Also the resistance rankings for oat genotypes were different in field compared to greenhouse, which can be seen from Fig. 2c and 2d and confirmed by significant interaction between oat genotype and experiment for both DON ($P<0.05$) and FIK ($P<0.001$, Paper II).

In field experiments, also traits other than DON, FIK and GC that affected FHB resistance were detected. Height (Papers II and IV) and earliness (Paper II) of an oat genotype had strong impact ($P<0.001$) on the DON accumulation of oats in field trials. The five hulless genebank accessions in the study were highly resistant against FHB in both greenhouse and field, but the hulled genebank accessions that were among the most resistant in greenhouse were not as resistant in field. One example is the genotype VIR7766. Other spikelet-related traits that were studied were flowering traits and these were found to vary significantly in oat and also influence on the degree of FHB resistance. The most cleistogamic and the most open flowering (OFL) oats found, accumulated both less DON than the genotypes with intermediate values in flowering traits (Paper IV). The rates of OFL and

anther retention (AR) had a highly negative phenotypic correlation (r=-0.85, P<0.01, Paper IV), i.e., a high rate of OFL leads to high anther extrusion (AE).

4.3 RESISTANCE RANKINGS

Nordic cultivars, breeding lines and selected genebank accessions had a large variation in DON, FIK and GC (Fig. 2) and in this study various degrees of resistance and susceptibility were found (Table 2). Table 2 lists example genotypes of contrasting resistance to DON accumulation. This information can be used for breeding purposes or for further research. The most promising lines were either hulless genebank accessions or breeding lines (Papers II and IV). The BLUEs for DON accumulation of the 16 genebank accessions were on average lower in both field and greenhouse experiments (P<0.001, Paper II) than the average estimates for the 40 cultivars and 348 breeding lines investigated. Similarly, eight older cultivars or accessions including Schenkenfeldener stood out with lower DON and T-2 mycotoxins from a panel of 25 oats used for studying the impacts of flowering traits to *Fusarium* infections (Paper IV).

Table 2. Contrasting oat genotypes highlighted from Papers II-IV. Resistance to DON accumulation in field conditions is categorized by the author from moderately resistant (MR) to susceptible (S) and intermediate moderately susceptible (MS)

Name	Resistance to DON accumulation	Reason for highlighting
AVEIA	MR	High rate of anther retention
BELINDA	MR	Average check cultivar in Norway
BOR03	MR	Lowest DON contents among hulled oats
BOR15	MR	Breeding line with multicomponential resistance
BOR31	MS	Rejected variety, the highest DON in greenhouse
MIRELLA	S	Susceptible in field
NIKLAS	MR	Most popular Finnish cultivar
OBELIX	S	Susceptible in field
ODAL	MR	One of the most resistant Norwegian cultivars
ROCKY	S	Susceptible in field
SCHENKENFELDENER	MR	High rate of anther extrusion
SYMPHONY	MS	Moderately susceptible cultivar
VIR11012	MR	Hulless and the most resistant accession
VIR6963	MR	Brown-hulled genebank accession
VIR7766	S	Susceptible in field, resistant in greenhouse
VIR7934	MR	Resistant genebank accession with white hull
VIR8479	MS	Moderately susceptible genebank accession

5 DISCUSSION

5.1 AVAILABILITY OF INFORMATION ON FHB RESISTANCE IN OATS

The following conclusions were made in our review article, titled "Evaluating resistance against *Fusarium graminearum* in oats" (Paper I). The basic information on FHB resistance in oats was found to be available and can be utilised for plant breeding purposes. Several oat genotypes were named as potential resistance sources for prebreeding, e.g. from the Far East East (Loskutov et al. 2016, Gagkaeva et al. 2013) or from North and South America (Bjørnstad et al. 2017, Tekauz et al. 2008). The landraces VIR7766, VIR6963, and VIR8479, which were cited as the most resistant hulled landraces by Gagkaeva et al. (2013), were included in the experiments in Paper II.

There is evidence that consistent selection with the methods available can be used to improve resistance levels in oat cultivars (Tekle et al. 2018). These methods, including repeated measurements of DON and GC from seeds harvested from spray- and spawn-inoculated nurseries, are time-consuming and expensive. Moreover, a lot of replication is necessary to handle environmental variance. Despite appropriate methods for the experiments of this thesis were determined, investments in more efficient phenotyping, novel genomic selection tools, and pre-breeding programs are necessary in future. Resistance breeding could be speeded by methods that quantify resistance parameters prior to harvesting or by development of low-cost laboratory analysis methods.

Traits correlating with FHB were also found that potentially supplement selection or increase efficiency in genomic prediction. Suggested components of resistance in cereals, associated traits or potential traits, and their mechanisms are presented in Table 3, which is modified from Paper I. Some of these traits were further studied in this thesis. An oat cultivar with high levels of resistance against FHB should reach heading and maturity under conditions that are not disease conducive, its stem should be robust and not too short, and its panicles should have a low disease incidence, low mycotoxin content, and a high number of heavy grains with good GC even under strong disease pressure. Breeding of ideotypes of a resistant oat cultivar is complicated when using resistance sources from landraces and crop wild relatives. Several backcross generations are needed to filter out the undesired genes that can result in inferior agronomy. Furthermore, after every backcross, all individuals should be phenotyped from the progeny in order to identify the resistant ones.

Table 3. Summary of commonly accepted FHB resistance traits in cereals (modified from Paper I). The table is divided into mechanisms and measurable traits for oats by the authors of Paper I. If applicable the mechanisms are further divided into traits that are continuously expressed or that require interaction. The information was gathered from: Aamot et al. 2012, Bjørnstad et al. 2017, Bjørnstad and Skinnes 2008, Skinnes et al., 2010, Boutigny et al. 2008, Gagkaeva et al. 2013, Martinelli et al. 2014, Mesterhazy 1995, Mesterhazy et al. 1999, Miller et al. 1985, Schroeder and Christensen 1963, Tekle et al. 2012, Tekle et al. 2013, Tekle et al. 2014, Tekle et al. 2018, Walter et al. 2010.

Types of resistance	Mechanisms	Traits and potential *) traits
Initial infection (type I)	Avoidance mechanisms, morphological and chemical barriers	Cleistogamy, height, earliness, lodging, level of anther extrusion, surface structure *, absence of hulls, lemma colour, phytochemicals *
	Defence against cell wall penetration: antifungal compounds, cell wall fortification	Incidence, diseased/ damaged kernels, fungal biomass within floret, defence related proteins, cell wall thickening*, and spectral changes at infection site*
Spread of infection (type II)	Morphological and chemical barriers	Panicle size and density, phytochemical compounds *
	Similar to type I in addition detoxification (type V)	Symptom severity, fungal biomass and fluorescence * within panicle
Toxin accumulation (type III)	Slowing the infection by type I or II resistance may reduce the total toxin accumulation. Detoxification, toxin transportation, anti-oxidation, prevention of toxin synthesis.	Toxin and masked mycotoxin contents directly (chromatography, ELISA, rapid tests, or indirectly with imaging technology (VIS/NIR).
Kernel infection (type IV)	Types I, II and III protect also kernels. Kernel structure *	Kernel structure * and biochemical constitution *
	Defence proteins and fortification, induced resistance	Diseased/damaged kernels, germination capacity, (hyper) spectral imaging, proportion of infection when inoculated post anthesis(?)
Tolerance (Type IV)	Compensation capacity, antioxidant content	Water and nutrient use efficiency*, high yield*
	Antioxidant production, allocation of resources, detoxification	Panicle yield or 1000-kernel weight versus disease severity or toxin content

5.2 METHODS FOR PHENOTYPING RESISTANCE

Finding suitable methods for phenotyping resistance against Fusarium head blight and DON accumulation in Finnish oat breeding was one of the main goals of the project “Development of breeding technologies for oat Fusarium resistance”. The major part of the data for this thesis was gathered during the project.

DON estimates based on 13 field experiments were consistent with the results of a large analysis made previously by the Finnish Cereal Committee (VYR) from grain sample data, provided by the cereal trade and industry (Finnish cereal committee's website, accessed 6.2.2019). The cultivars - Rocky, Obelix and Mirella - that were found most susceptible in Paper II were also the most susceptible samples collected from farmers' fields. Similarly, the cultivars with the lowest DON estimates performed well also in VYR's listing, i.e. cultivars such as Niklas, Alku and Belinda, did not show high DON medians in that study.

Field testings in this study relied mainly on spawn inoculated nurseries. There, an artificial inoculum was spread on the ground before anthesis mimicking the natural infection by *F. graminearum,* whose primary inoculum source is the crop residue (Dill-Macky & Jones 2000, Hofgaard et al. 2016b). In addition, the conditions were kept conducive to disease by increasing moisture through mist irrigation during evenings, which favours ascospore release (Sutton 1982, Paulitz 1996). According to Paper I, many of the latest resistance evaluations in oat relies on spawn inoculum (Tekauz et al. 2004, Gagkaeva et al. 2013, Bjørnstad et al. 2017, Tekle et al. 2018). With several replicated trials over space and time, these evaluations have been able to make rankings that can distinguish highly susceptible and moderately resistant oat genotypes. According to statistical power calculations in Paper II, the least significant difference was found to pass below 3000 µg/kg, when the number of field experiments behind the estimates rose from three to four. This is completely in agreement with the results of Tekle et al. (2018), who got least significant differences of 3000 µg/kg based on a minimum of four field experiments.

Fewer genotypes were tested in the greenhouse experiments than in the field; thus, our data is not optimal for comparing field and greenhouse experiments. Potentially resistant genebank accessions showed high resistance levels in the greenhouse. However, there was also plenty of inconsistencies in the rankings of genebank accessions, breeding lines or cultivar estimates between the field and greenhouse. These differences can be explained by the escape mechanisms that play a role in field. Theoretically, in the greenhouse, height or earliness should not have an impact on disease infection severity. Greenhouse spray inoculations can be done according to the plants developmental stage and height and humidity in controlled temperature conditions. Large differences in these traits can, however, have undesired impacts on disease development. For example, higher plants are closer to the warm lights. In addition, during spring or autumn the rapid change in day length over few weeks can have impacts on the light, temperature and moisture conditions inside the greenhouse. Nevertheless, the temperature, moisture and light conditions are considered more stable in greenhouse than in field.

The high correlations found between DON and FIK (Papers II, III and IV) suggests that in suitable conditions the initial infection often directly leads to

accumulation of DON mycotoxin. Paper III emphasizes the role of type I resistance in oats which is resistance against the initial infection (Schroeder & Christensen 1963). In wheat research, type II resistance is commonly assessed by inserting *Fusarium* spores inside a spikelet by point inoculation and measuring the spread of symptoms after inoculation (Mesterhazy 1995). The panicle structure of oats, however, constrains the spread of symptoms and thus Langevin (2004) did not find differences in type II resistance of oats. The cell level mechanisms against the initial infection and the spread of infection should be somewhat similar including morphological and chemical barriers at the cell wall and active defence through cell wall fortification, antifungal compounds and maybe also detoxification of mycotoxins at least in the case of wheat FHB (Walter et al. 2010). The point inoculation method used in Paper III was applied from a study on wheat and barley by Kumar et al. (2015). There, in addition to symptom spread, a clear difference in fungal biomass was found between different genotypes. However, the two oat genotypes investigated with large differences in their DON accumulation and proportion of FIK in greenhouse experiments did not show any difference in their fungal biomass accumulation. At least in this case, *F. graminearum* was able to spread as efficiently within the tissue of the susceptible cultivar as within the tissue of the presumably moderately resistant oat.

In point inoculation, individual spikelets got heavily infected during 6 days, which also suggests that the resistance against infection spread is mainly affected by the panicle structure. This was also predicted by Tekle et al. (2012), who estimated that established infection in the primary floret may make the secondary and other later flowering florets more vulnerable. Typical *Fusarium* infected oat spikelets lose their chlorophyll under field conditions, which suggests that the infection spreads or kills all florets within a spikelet. The study in Paper III was made only by two different oat genotypes and further research with additional oat genotypes would be valuable in determining whether oat tissue has ability to resist this kind of infection. The genotypes compared in Paper III, had high difference in their resistance response when spray inoculated in greenhouse, but in field conditions the difference was not significant (Paper II). The different distributions in DON accumulation and FIK in field and greenhouse, and differences between GC and DON rankings in our research (Paper II) and in other investigations (Tekle et al. 2018) suggest that there are several mechanism at work. Thus differences in infection spread at tissue level may be detectable in some oat genotypes. Also the strength of the inoculum and the time point of comparison need to be considered. Only one concentration of inoculum was used in the point inoculation study, which may have resulted to infection pressure so high that small differences in resistance against infection may have disappeared. Inoculation by a slower growing pathogen *F. langsethiae* (Divon et al. 2019) did not cause any weight reduction by 6 dpi.

Significant genotypic correlations between the estimates of FIK and DON in sets of genotypic estimates based on several experiments (Papers II, III and IV) suggest that type I resistance is a highly important determinant for resistance in oats. Considering the constant release of spores from heading to yellow maturity in our spawn inoculated field experiments, it is not surprising to have majority of grains at least superficially infected. This leads to low rate of differentiation between genotypes and reduces the ranking power of FIK. A greenhouse study conducted by Tekle et al. (2013) demonstrated that timing of inoculation can have substantial impact on the relationship between DON accumulation and the level of infection. It is suggested that anthesis is the most susceptible time of inoculation. The field research of Gagkaeva et al. (2013) also found clearly weaker correlation between mycotoxins and *Fusarium* infected seeds than between mycotoxins and fungal biomass. The use of a quantitative PCR method for determining the amount of fungal biomass from field samples and consequently severity of infection could produce more reliable data than proportion of FIK. Further investigations in inoculated environment are, however, needed, since the reported correlations between fungal biomass and mycotoxin production vary a lot between different samples and environment (Gagkaeva et al. 2013, Yli-Mattila et al. 2017).

The correlation between DON and GC in our research was similar to the results obtained by Tekle et al. (2013 and 2018), who determined that in oats DON can restrict seedling growth but the loss of GC is more dependable on the fungus. The infections occurring at anthesis often killed the germ and led to accumulation of mycotoxins, whereas later occurring infections were able to weaken GC but not to cause significant mycotoxin accumulation (Tekle et al. 2012, Tekle et al. 2013). The average GC and the GC ranking of oat genotypes was affected by the level of DON (Paper II), which suggests that there are either differences in tolerance to infection and DON accumulation or differences in susceptibility to lose GC once infected. The resistance against kernel infection is one of the resistance types in cereals (Mesterhazy et al. 1999) and it has been measured as a resistance component in Norwegian FHB screenings of oats (Tekle et al. 2013, Tekle et al. 2018). The results of Paper II further encourage the use of this trait in resistance screenings of oats. According to data of Paper II, cultivars such as Akseli, Niklas, Eemeli, and Belinda, are potential sources for resistance against kernel infection.

5.3 TRAITS CONTRIBUTING TO FIELD RESISTANCE

Figure 3. Hulless oats (left) and brown/black oats (right) on August 2016 (soft dough stage).

Passive resistance can be determined as resistance that is not dependent on the detection of the pathogen. Tekle (2014) describes passive resistance mechanisms against FHB being morphological traits affecting inoculum deposition or establishment of infection on cereal heads. Some of these traits are also referred to as escape mechanisms or evasion factors. As has been shown, the results between different phenotyping methods are not consistent in all oat genotypes. Type I resistance in oats can be highly contributed by several escape mechanisms that can have high impact on the severity of FHB especially in field conditions. The agronomic and morphologic characters potentially affecting FHB in oats are described and discussed in the literature review (Paper I). In this chapter, these are reflected into the results of our research.

The higher FHB resistance level in hulless accessions (Fig. 3) is in agreement with other research results (Gagkaeva et al. 2013, Tekle et al. 2018), where hulless oats have also been more resistant than others. Most of the mycotoxins accumulate on the hulls which are also called as palea and lemma, since these are the first parts to be colonized by *F. graminearum* (Tekle et al. 2012). The genotypes that lose hulls during the threshing have less mycotoxins than typical hulled genotypes where the hulls need to be removed mechanically. Loss of hulls at maturation, may not however, stop optimally timed *Fusarium* infections from killing the embryos and thus the yield impact of FHB in hulless oats may not be different compared to hulled

cultivars. The yield impact of FHB may actually be greater due to indeterminate flowering of hulless oats.

Four of the resistant genebank accessions in Paper II were also characterized by brown hull colour (Fig. 3) and the average DON content was lower ($P<0.001$) than the average for the rest of the oats in Paper II. Dark-colour in hulls has been connected to resistance against *Fusarium* infection both a long time ago (Rainio 1932) and also recently (Loskutov et al. 2016). In black barley the similar resistance was associated to *Fusarium* growth inhibiting compounds including phenolics (McKeehen et al. 1999), lignin (Siranidou et al. 2002) and flavonoids (Skadhauge et al. 1997). Black or brown hull colour was associated with resistance on oats (Rainio 1932) and in barley (Choo et al. 2015). Neither, the hulless nor the black or brown hulled genotypes are interesting for the current market (Leena Pietilä, oat breeder at Boreal Plant Breeding Ltd., pers. discussion) and thus it was decided not to continue the field research with these genebank accessions.

F. graminearum spores and hyphae are susceptile to desiccation in dry air and sunlight, despite of their own protective pigments (Gambaza et al. 2018) and hydrophobins (Quarantin et al. 2019). Therefore the spores on the air or the hyphae growing on the thick external surfaces of florets need to reach for the soft inner surfaces of palea and lemma as well as anthers and developing caryopsis. In oats, the fungus typically enters via the floret mouth (Tekle et al. 2012). Thus, differences in flowering morphology can act as passive resistance mechanisms.

At anthesis, the opening flower lets in the airborne spores. After anthesis, anthers that are retained inside the flowers start to degrade and provide dying tissue where *Fusarium* fungi can proliferate (Tekle et al. 2012, Divon et al. 2019). Tekle (2014) suggested in her thesis that further research should investigate the importance of AE as a passive resistance mechanism. She also underlined that the flowering biology of oats should be further investigated. Cleistogamic and open flowering oats were searched among 50 candidates by estimating OFL and AE and measuring AR. These three flowering traits correlated relatively well with each other, but AR can be recommended as the most precise and reliable method, although it is also rather time consuming to assess. A cleistogamous oats was found and genotypes of high rate of OFL were also named. The highest AE rates were found among older cultivars and accessions. Similarly, Stråbø (2015) observed a clear decline in AE among modern Nordic cultivars when compared to older cultivars. In that study, the highest AE was similar to the highest AE in paper IV.

AE was expected to have an impact, since oats are most susceptible during flowering (Tekle et al. 2012, Xue et al. 2015) and AR is commonly known to affect resistance against FHB and DON in wheat (Strange and Smith 1971, Skinnes et al. 2010, Steiner et al 2019). In barley, the cleistogamous genotypes are less susceptible than open flowering genotypes during flowering but they become more susceptible later when the developing grain pushes the degrading anthers so that they become visible and available for

the *Fusarium* spores and mycelium (Yoshida et al. 2007). In our study, the most cleistogamous and the most open flowering accessions Aveia and Schenkenfeldener, respectively, had similarly low DON levels, but they had considerably high differences in their GC, which may be due to anther extrusion, since later infections are shown to have greater impact on GC than toxins (Tekle et al. 2013). Only a part of genetic variation of FHB resistance can be explained by variation in AR, thus more precise evaluation of effects by AR on mycotoxin content is required. This needs to be done with populations that have high range in AR, but less variation for other traits that can have impact Fusarium infection such as plant height and flowering date as in a recent wheat study (Steiner et al. 2019).

Weather conditions favourable for *Fusarium* species are shown to promote mycotoxin production in oats (Hjelkrem et al. 2016, Kaukoranta et al. 2019) as well as T-2/HT-2 production in UK (Xu et al. 2014). Thus, the maturity of the host genotype can offer an escape mechanism that is shown to affect the severity of FHB in oats (Loskutov et al. 2016, Bjørnstad et al. 2017, Tekle et al. 2018, Rainio 1937). The optimal phenology depends on the agricultural environment. For example in Norway the early maturing genotypes were more susceptible than late maturing (He et al. 2013, Bjørnstad et al. 2017) and also in the experiments of Paper IV that were made in Germany (Paper IV). In Finnish conditions, the early genotypes were less susceptible than late maturing genotypes (Paper II) and this has been also found in other research in Finland (Parikka et al. 2008) and Russia (Gavrilova et al. 2008). In Finland, early flowering can occur before the warmest part of the growing season and it can make early genotypes less vulnerable to the combination of warm and moist weather promoting *F. graminearum* infections. Late maturing oats can also face rainy autumn weathers that can promote accumulation of DON toxin especially when they are suitably timed with development of oats and optimal temperature (Hjelkrem et al. 2016, Kaukoranta et al. 2019). The growing seasons are, however, variable and the development of oats is fast in the long days of high latitudes and thus the maturity is not likely to resolve the entire FHB problem in oats.

Autumn weathers during harvesting in, for example, Norway or UK, are usually warmer than in Finland (Kaukoranta et al. 2019). Slightly different rankings of cultivars Belinda and Symphony in our experiments compared to Norwegian experiments may be explained by differences in climatic conditions. Additionally, earliness may partly explain why the rejected variety BOR31 was highly susceptible in greenhouse, but only moderately susceptible in field. BOR31 was one of the earliest genotypes of our experiments (Paper II). Contrastingly, there were also genotypes such as BOR03 that could clearly resist toxin accumulation in field (Paper II), despite being among the latest maturing. This genotype was not tested in greenhouse and it could be interesting to study its type I resistance by seeing if the DON accumulation in this genotype is connected with infected kernels or not.

Short plants or very high plants with late maturity and tendency to lodging accumulated more *Fusarium* infections and DON than average genotypes in Finland (Paper II). The only dwarf genotype in our inoculation research, Troll, had more mycotoxins than the other 24 genotypes within the same panel (Paper IV). Despite being closer to the source of inoculum in the soil and suffering from a higher inoculum density in the air (Manstretta et al. 2015), short plants may also have more disease conducive microclimate in field nurseries. Their panicles are closer to the leaves of taller genotypes, which can both act as bridges for conidia as well as concentrate moisture. Height is also regarded as component of type I resistance in wheat (Lu et al. 2013) and it is shown to contribute *Fusarium* infection rankings in genebank accessions (Loskutov et al. 2016). The impact of height and earliness should be considered in future screenings and analyses by either, selecting sets of similar height and earliness material as was done by leaving Troll out from the analysis in Paper IV, or using earliness and height as covariant (Tekle et al. 2018, Bjørnstad et al 2017).

5.4 FUSARIUM HEAD BLIGHT RESISTANCE IN OAT

At the beginning of this PhD project there was a clear lack of information regarding the level of resistance in the current oat cultivars and breeding population in Finland. Moreover, there was no evidence on how the promising genebank lines would perform in Nordic conditions. This chapter describes and discusses the variation in FHB resistance and accumulation DON mycotoxin found in Papers II, III and IV and reflects it with literature gathered in Paper I.

The screenings by the N. I. Vavilov All-Russian Institute of Plant Genetic Resources (Gagkaeva et al. 2013) found no significant differences between cultivars and landraces when screening their genebank collections. In this thesis, the cultivars were generally new and the genebank accessions in Paper II were selected based on available resistance data. Thus, a wider gap between these groups of genotypes can be assumed, and a significant difference in resistance was also expected. In the field, the ranges of DON estimates were, however, quite similar between the cultivars, breeding lines, and hulled genebank accessions (Table 3 in Paper II). Moreover, if the hulless lines are excluded, there was no genebank accession that could exceed the most resistant genotypes within the Nordic breeding pool. Considering that FHB resistance is a quantitative trait and FHB can have yield impacts, it is easy to agree with Bjørnstad et al (2017), who suggested that breeding for yield in moist Nordic conditions has already made some progress against FHB. Nevertheless, the good performance of genebank accessions in greenhouse and the vast genetic diversity in landraces compared to modern cultivars (He & Bjørnstad 2012) both encourage

examination of genebank accessions more in detail. Consequently, new pre-breeding efforts may be started.

Despite the genebank accessions from N. I. Vavilov All-Russian Institute of Plant Genetic Resources being generally more resistant than other genotypes, some genebank accessions turned out to be moderately resistant in greenhouse to moderately susceptible in field (Paper II). For example, VIR11012 had the lowest estimate for DON accumulation in greenhouse (462 µg/kg). This was clearly better than any other genotype, but in the field it had an estimate of 7525 µg/kg, which was at the same level as the estimate for the best cultivar. Hulled genebank accessions VIR7766 and VIR8479 accumulated relatively more DON in field than in greenhouse (16037 vs. 4640 µg/kg and 14362 vs. 5148 µg/kg, respectively), whereas VIR6963 behaved quite similarly in both environments (10569 vs 4462 µg/kg). These results suggest that pre-breeding would be required to see whether the resistance of a specific accession can be integrated in the Nordic oat genepool successfully. The biggest challenge in the application of resistance from genebank accessions is that the genetics of the resistance is yet unknown. As long as the genes behind the resistance are not associated with markers, comprehensive phenotyping has to be made in every backcross generation. Thus, the use of resistance existing already within the breeding population may be less cumbersome approach to improve FHB resistance in oats in the near future.

In the results of Paper II and in Figure 2, clear relationships between the resistance traits (DON, FIK and GC) can be seen, but also interesting outliers can be identified. For example, the most DON resistant breeding line in field was BOR03 with DON estimate of 5007±2978 µg/kg (mean±S.E.) but it had relatively weak GC estimate of 58±8.5 % and rather intermediate FIK rate (57±8.6 %). Contrastingly, breeding line BOR15 had almost similar DON (6000±3294 µg/kg), but with clearly better GC estimate of 74±8.3 % and lower FIK estimate (46±8.4%). These different responses in different traits indicate that there may be different resistance mechanisms in these phenotypes. The Finnish cultivar Niklas had the best DON estimate among cultivars in field (7204±3703 µg/kg) and also relatively low estimate for DON in greenhouse (5792±3515 µg/kg). One of the most DON resistant oat cultivars in Norway (Tekle et al. 2018), Odal, was also moderately resistant to DON in our experiments with a DON estimate of 10335±4846 µg/kg in field and average DON estimate in greenhouse (8430±1823 µg/kg). The most susceptible accessions in field were the cultivars Rocky, Obelix, Mirella with DON estimates ranging from 17490±5540 to 20138±5881 µg/kg). These three also had GC estimates below 55%. Mirella was also associated with high mycotoxin content and low GC in Norway (Tekle et al. 2018). The most susceptible genotype in greenhouse, BOR31, was however; only moderately susceptible in field experiments with a DON estimate of 13553±4852 µg/kg, whereas in greenhouse its DON estimate was as high as 22059±3163 µg/kg.

The rankings of cultivars across evaluations can be used as cross-validation but also to determine genotype×environment interaction. Cultivars Mirella and Odal rank similarly between Paper II and Norwegian screenings but for example cv. Symphony had less DON accumulation in our conditions (Papers II and IV) than in Norwegian conditions (Tekle et al. 2018). In addition to Finnish conditions, Symphony was also studied in Germany (Paper IV) and the results from these trials were similar to the results obtained in Finland. In the study made in Germany, however, the most susceptible cultivar for accumulation DON and T-2/HT-2 toxins in a panel of 25 oats was Bessin, which is the susceptible control in Norway (Tekle et al. 2018, Bjørnstad et al. 2017). Bessin has been also found to rank as susceptible in Russian FHB resistance screenings where also Belinda was intermediate and Odal resistant (Gagkaeva et al. 2017). Unfortunately, Bessin was not included in the study of Paper II and thus there is no estimate for its performance in Finnish conditions. Since genotype×environment interactions are likely to occur due to, for example, different weather conditions and earliness requirements of the sites, the use of common check cultivars can be recommended for phenotyping studies to better understand these mechanisms. Phenotypic effects of QTL for resistance are often incidental, showing only in certain experiments even with same mapping populations (Niks et al. 2011).

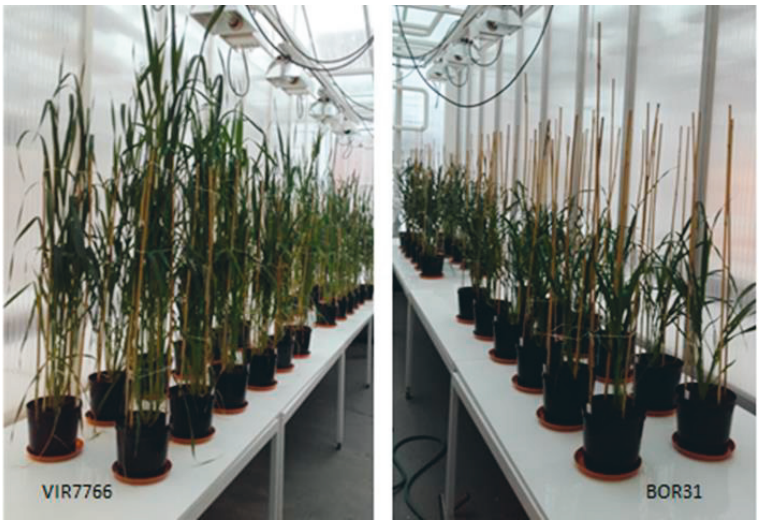

Figure 4. Contrasting genebank accession VIR7766 and rejected variety BOR31 grown for point inoculation experiment in a greenhouse environment. Settled for the image, originally mixed with each other.

The comparison of the rejected variety BOR31 and the genebank accession VIR7766 can serve as an example how different resistance

components can become important in different conditions (Fig. 4). These two genotypes were inoculated by all three methods (spawn, spray, point) with very contrasting results. BOR31 was clearly more susceptible than VIR7766 in spray inoculated greenhouse experiments measured by both DON accumulation and by FIK incidence (Papers II and III). However, the results from field experiments inoculated mainly by spawn methods found these genotypes to be rather equally susceptible and VIR7766 had actually higher DON estimate than BOR31 (Table 3, Paper II). Moreover, the results from point inoculations in Paper III showed that both of the genotypes similarly accumulated *F. graminearum* biomass and lost their fresh weight at spikelet level (Paper III). These results indicate that once established the infection spreads and leads into certain accumulation of DON in a spikelet, but the oat genotype has an ability to resist the initial infections and in field conditions this ability is contributed by different factors than in greenhouse.

In short, it seems that these two genotypes differ mainly in their resistance against the initial infection (type I), which can be contributed by the following differences in these two genotypes. VIR7766 is one of the highest and most late maturing accessions whereas BOR31 was the earliest genotype in these investigations and also relatively short. BOR31 is a cultivar, whereas VIR7766 is a dark-hulled landrace accession. According to a preliminary study (data not shown), BOR31 also has relatively high level of AE and low AR among our core set of oat genotypes. High rate of open flowering could actually increase its susceptibility in spray inoculations at greenhouse and protect it from constantly released spores in the field.

The FHB resistance in oats is a puzzle that cannot be solved if only one piece is considered. In Figure 5, a suggestion of different pieces of resistance is made based on the results gathered in Papers I-IV. This figure underlines that different mechanisms behind the variation found in oats should be considered. Some of the statements (Fig. 5) are still incomplete. For example the mechanism that is protecting from DON accumulation in BOR03 and mechanisms increasing susceptibility in Rocky, Mirella and Obelix require further research in greenhouse environment. These different pieces of the puzzle can be highly valuable, when they are used together. For example, genomic prediction of DON accumulation could be enhanced by GC and FIK data and perhaps also by data from different environments.

More pieces of this puzzle are likely to be identified when more phenotyping is made in future. One should always remember that a phenotype is only a reflection of genotype in the specific environment where it has been observed. The precision of phenotyping is dependent on the number of environments where it has been done. Thus determining each and every factor influencing on the resistance of a specific genotype would require phenotyping in limitless set of environments.

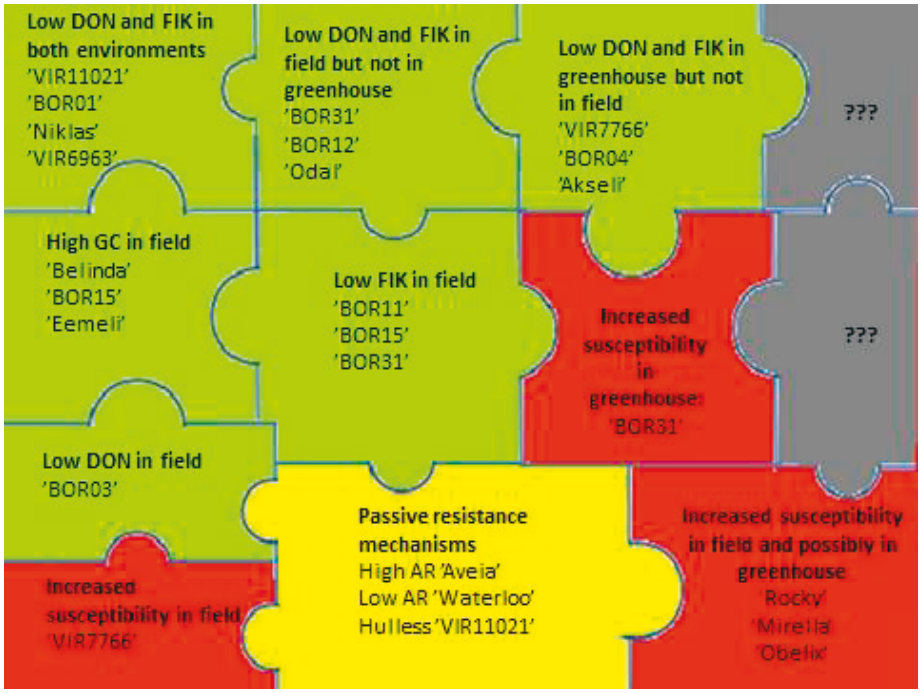

Fig. 5. The puzzle of Fusarium head blight resistance components in oats. Each piece of the puzzle resembles a mechanism that can be suggested based on this research data and gives also examples of genotypes that manifest this mechanism. Empty gray pieces resemble mechanisms that remain unknown. Green pieces describe resistance and red susceptibility. Passive resistance mechanisms are marked with yellow since these may promote either resistance or susceptibility depending on the circumstances.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

Several methods to phenotype FHB resistance in oats were evaluated. From a breeding perspective and for cultivar evaluation purposes, field trials give more comprehensive and applicable resistance rankings than greenhouse testing. The latter can, however, be used to produce information on single resistance mechanisms due to more controlled environmental conditions in the greenhouse than in the field experiments. Resistance associated traits DON, FIK, and GC were found to partially correlate, but the numerous outliers that either rank as weak in DON but good in GC, or vice versa, indicate that these also reflect different dimensions of resistance. Thus, at least two of these three or equivalent methods should be used in phenotyping.

All types of oat germplasm, including cultivars, breeding lines and genebank accessions, were found to include genotypes with different degrees of susceptibility to DON accumulation, *Fusarium* infections and loss of GC under infection. Thus, it is important to phenotype different types of material in order to identify new sources for resistance breeding. Constant selection against FHB within the breeding population would also shift the population towards lesser susceptibility.

Resistance against the initial infection (type I) was found to be a highly important character for host plant resistance in oats. Type I resistance can be enhanced or reduced by morphological (flower structure and flowering physiology) and phenological (height, earliness) traits in field, whereas in the greenhouse it more purely reflects the plant's active mechanisms working against infection and infection spread. In addition, colour and dropping of the hulls, both extremely closed and extremely open flowering, increased passive resistance in oats. The proportion of retained anthers was shown to sort out oat genotypes with different rates of flower opening. Thus, AR can be used to select for lower mycotoxin content.

Great challenges for the breeding of FHB resistant oats come from the sizes of the breeding populations and from the need of expensive repetitions and analyses similar to those of this study. Thus, the development of genomic prediction tools for breeding FHB resistance in oats would be highly valuable since these would in the long run reduce the need of expensive phenotyping and make it possible to improve the entire breeding population. Even genomic selection requires efficient and intensive phenotyping when the model is being developed, but when an accurate model is available the phenotyping can be reduced (Jannick et al. 2010).

The need for further investigations to make better generalization of our results in either larger sets of genotypes that are more representable such as in the case of spikelet infection (Paper III) or more advance material with

less variation such as in the case of flowering traits (Paper IV) arose. If genebank accessions are found to contain resistance mechanisms lacking in the modern gene pool, then pre-breeding programs could have highly positive impacts to FHB resistance.

The availability of anthers was shown to impact on the severity of FHB in oats, but these investigations focused on the rate of flowers open or on the amount of anthers extruded, even if the duration of flowering within a certain oat genotype could easily be as affective a trait. Duration of flowering is very laborious to be quantified by observation, but in the future, automated imaging by stationary cameras could result to identification of variation in the duration of flowering within oat genotypes. We also need data on the importance of flower opening under greenhouse conditions. Imaging technology can proof itself useful also in replacing existing techniques by more efficient and cost effective technology such as replacing DON analysis with hyperspectral imaging (Tekle et al. 2014).

The lack of resistance genes conferring strong resistance could also encourage researchers and breeders to use new plant breeding technologies to modify oat plants more resistant to FHB. For example *Fhb1* QTL in wheat is suggested to result from either gain or loss of function after a deletion mutation (Su et al. 2019, Li et al. 2019). Systems such as CRISPR-*Cas9* could be applied in the introduction of similar mechanisms in oats. Naturally this would require public acceptance and the duration of this kind of resistance should be considered critically. There is, however, no evidence on breaking of FHB resistance, even in wheat. Maybe this is the positive aspect of a disease with multiple and highly variable causal agents.

The functions of currently detected resistance mechanisms in oat are also unclear. For example, is the resistance against initial infection - after the passive resistance mechanisms are filtered out - related to cell walls or on specific resistance proteins? Or is the lower DON content in specific genotypes a result from detoxification, degradation, transportation or something else? Comparative genomics and RNA sequencing of inoculated and healthy spikelets at different time points could help to reveal this in future. Perhaps, there are also susceptibility genes. Studying the susceptibility of rejected variety BOR31 could provide interesting information on how *Fusarium* infection invades oats.

The grain yield impacts of FHB (Kiecana et al. 2002) should be compared in oat germplasm for better dissemination of different resistance mechanism in oats. The empty kernels resulting from very severe and well-timed infections are suggested to cause misinterpretation of resistance (Bjørnstad & Skinnes 2008, Bjørnstad et al. 2017). Estimation of empty kernels did not, however, clearly improve DON estimates in a study by Bjørnstad et al (2017). Better understanding of the factors causing the differences between field and greenhouse environment or a system, where a comparable yield from uninoculated and inoculated field plots could be achieved, may answer this question.

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